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1	Comparative biometrics of British Marsh Tits Poecile palustris and Willow Tits P. montana
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15	Running head: Biometrics of Marsh Tits and Willow Tits
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29 ABSTRACT

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Biometrics are commonly used to compare bird species. For British Marsh Tits and Willow Tits there are few biometric data from birds of known age and sex, despite their value for population analyses in estimating the proportion of males and females in samples. Comparing measurements between the two species could also aid identification and the monitoring of these declining species in Britain.

We present biometrics for a large sample of British Marsh Tits of known age and sex, 36 and new data for British Willow Tits, which act as reliable reference material. Overall, adults 37 of both species were larger than first-years and males were larger than females, but not 38 among first-year Willow Tits. Marsh Tits were slightly larger and heavier than Willow Tits, but 39 40 Willow Tits had proportionately longer tails. Discriminant analyses produced new equations 41 for separating the species based on wing length and the measurement between the shortest 42 and longest tail feathers. Probabilities were generated for estimating Marsh Tit population 43 structure from samples of ringing data, but there was a greater overlap between sexes in 44 Willow Tit measurements. We conclude by discussing issues of measurement accuracy and 45 consistency in the collection and analysis of biometric data.

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47 Keywords: measurements, subspecies, taxonomy, wing length

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56 Descriptive taxonomy is a foundation of ornithology, comparing the physical measurements of birds in the classification of species and subspecies. Standard reference works give some 57 biometrics for all bird species that occur regularly in Europe (e.g. for the tits (Paridae) see 58 Cramp & Perrins 1993, Glutz von Blotzheim & Bauer 1993, Gosler & Clement 2007). In 59 60 many instances, however, these texts also highlight a lack of data, even for some common and widespread species. The bird measurements reported in these reference works typically 61 include wing length, which is the most consistent measurement of body size of passerines 62 63 (Gosler et al. 1998), and also tail length and body mass (weight). Sample sizes are often 64 small (fewer than 30), with little information on the age class of samples (adults or juveniles). In addition, many such measurements were taken from skins, which can differ from those of 65 66 live birds due to shrinkage (Haftorn 1982, Svensson 1992).

Published reference data for biometrics are particularly inadequate for two formerly 67 68 common, but now much declined, species in Britain that are each represented by a localised subspecies: the Marsh Tit Poecile palustris dresseri and Willow Tit P. montana kleinschmidti 69 70 (Broughton 2009). Both species are 'red-listed' and of conservation concern in Britain due to respective declines in abundance of 74% and 92% between 1967 and 2013 (Robinson et al. 71 72 2015). Cramp & Perrins (1993) contains full summary statistics (mean and standard deviation and/or range) for the length of the wing, tail, bill and tarsus of just 12 male and ten 73 74 female British Marsh Tits, all taken from skins, with the range of body weights for seven birds. For British Willow Tits, Cramp & Perrins (1993) gives biometrics from the skins of up to 75 12 males and 6-50 females, but the average body weight of only 20 birds (all unsexed). For 76 both species no distinction was made between juveniles and adults, despite age being an 77 important factor in wing length (Broughton et al. 2008a, Hogstad 2011). 78

Further summary data of Marsh Tit wing lengths have since been published, including 89 sexed birds from Nottinghamshire (du Feu & du Feu 2014) and 230 birds aged and sexed birds in Cambridgeshire and Oxfordshire (Broughton et al. 2008a). Summary statistics of wing and tail length have also been published for 1147 unsexed Marsh Tits from across Britain, which demonstrated that there was no regional variation in these biometrics

(Broughton et al. 2016). For British Willow Tits, Scott (1999) reported wing length
measurements for nine birds and Robinson (2015) gives maximum chord values of 56-63
mm for 1418 birds in the BTO ringing database, with 59-66 mm for 2628 Marsh Tits, but du
Feu & du Feu (2014) have highlighted the large variation in this dataset which may indicate
some error.

Accurate reference biometrics for British Marsh and Willow Tits are particularly valuable, as this species pair can be difficult to separate, even in the hand, and differences in morphology may help ringers with identification (Redfern & Clark 2001, Broughton 2009). Wing and tail lengths have been used to compare Marsh and Willow Tits of other subspecies, either as a ratio ('tail/wing index', Eck 2006) or in discriminant analyses (Markovets 1999). For British birds, Scott (1999) found that the wing lengths of 14 Marsh Tits averaged significantly longer than nine Willow Tits, but there was substantial overlap.

Tail shape may also be helpful for identification, such as the 'tail tip difference' between the tip of the shortest, outermost tail feathers (T6) and the longest inner feather that forms the end of the tail (du Feu & du Feu 1996). This difference is generally greater for Willow Tits than for Marsh Tits, but Scott (1999) reported an overlap of 36% for British birds and Abe & Kurosawa (1984) found 54% overlap in Japan.

The number of tail feathers visible on the underside of the closed tail may also help identification (du Feu & du Feu 1996, Scott 1999); on Marsh Tits the outermost T6 is the shortest feather, with the longer T5 covering the inner feathers of almost equal length, giving a 'tail tip score' of two feathers clearly visible on each side (T6 and T5). On Willow Tits the tips of T6, T5, T4 and perhaps T3 are visible as a series of more evenly spaced steps (at least 1 mm apart) on the underside of the tail, giving a tail tip score of 3 or 4. The error rate for this test is unknown.

A further benefit of analysing the biometrics of British Marsh and Willow Tits is the estimation of population structure. Proportions of male and female Marsh Tits of a given wing length are reported in Broughton et al. (2008a), and such data could be used to approximate the population composition in other samples of ringing data. This approach could be used to identify any gender bias and offer clues to the causes of population
declines (Broughton & Hinsley 2015). Such information would be especially valuable for
British Willow Tits, for which data on sexing and population structure is generally lacking.

The aims of this paper were, firstly, to provide the most complete set of biometric data yet available for live Marsh Tits of known age and sex, with a consistent data quality. Additional biometric data were collected for British Willow Tits, including birds of known age and sex, to test whether measurements are useful for sexing birds. We also compare biometrics between the two species to test for differences which may aid ringers and analysts with identification.

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123 METHODS

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125 Marsh Tit biometrics

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A total 559 handlings of 448 individual Marsh Tits of known age and sex were made during all months of the year in 1993-2016. These comprised 386 birds from Cambridgeshire (Monks Wood: 52°24'N 0°14'W, and five woods within 5 km), with 34 birds from Oxfordshire (Wytham Woods: 51°46'N 1°20'W, Bagley Wood: 51°43'N 1°16'W), and 28 birds from Suffolk (Bradfield Woods: 52°10'N 0°82'E).

Birds were caught in baited traps or mist-nets and ringed with a BTO alloy ring and a 132 unique combination of colour rings (Broughton et al. 2008a). Ageing as first-years (EURING 133 codes 3J, 3 or 5) or adults (EURING codes 4 or 6) was based on prior ringing history, extent 134 of moult, and the presence/absence of distinctive juvenile greater coverts or tail feathers 135 (Amann 1980, Broughton 2010). Sexing was based on behaviour observed during fieldwork 136 (including the use of playback), such as 'courtship feeding', singing and aggressive 'gargle' 137 calls (males), and food soliciting, nest-building and incubation (females), or being paired with 138 139 a territorial bird of known sex (Broughton et al. 2008a, 2010). No sexing decisions needed to

be reversed on later observation (e.g. no birds recorded as singing 'males' were later foundas breeding females).

This behavioural sexing method was also validated against DNA sexing of 55 birds 142 based on blood samples taken under a Home Office licence during 2008-2011. Total 143 144 genomic DNA was isolated from whole blood which was taken from the brachial vein using a capillary tube and archived on Whatman FTA Classic Cards (GE Healthcare Life Sciences, 145 Maidstone). A single 1.25 mm disc was cut from the cards using a Uni-Core punch 146 (Whatman) and DNA extracted with FTA purification reagent (Whatman) and the ZR DNA-147 Card Extraction Kit (Zymo Research, Irvine, California) according to the manufacturer 148 instructions. The sex identification test employed the P8 (5'-CTCCCAAGGATGAGRAAYTG-149 3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3') primers (Griffiths et al. 1998) and PCR 150 amplification was conducted in a total volume of 10 µL using the PCR conditions reported by 151 152 Griffiths et al. (1998).

Measurements taken during handling of full-grown birds included wing length 153 (maximum chord to 1 mm precision), tail length (to 0.5 mm), the 'tail tip difference' 154 measurement between the ends of the longest and shortest tail feathers (to 0.5 mm, Fig. 1), 155 156 'tail tip score' as the number of tail feather tips visible \geq 1mm apart on the underside of the closed tail (one side only, Fig. 1), body mass (to 0.1 g using a calibrated Pesola spring 157 balance or electronic balance), and maximum tarsus length (to 0.1 mm). Standard 158 measurements were taken as described by Redfern & Clark (2001). Sample sizes differed 159 for each variable as not all measurements were taken in all years. The dataset included 230 160 wing length measurements from 182 Marsh Tits previously reported in Broughton et al. 161 (2008a), and additional measurements of tarsus were available from 30 birds in 2015 that 162 163 were unsexed.

A total of 111 Marsh Tits measured originally as first-years and later as adults were included in both age classes, because wing lengths differ significantly between these plumages (Broughton et al. 2008). For each individual, only the first measurements taken in each age class were included in analyses, and birds with obviously abraded flight feathers

(e.g. broken tips) were excluded. Measurements were highly consistent between sites, with
88% collected by a single experienced ringer (RKB), and comparisons between other ringers
(mostly trainers) were consistent.

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Figure 1. Schematic of typical tail morphology of Willow Tit (left) and Marsh Tit (right), with the end of the tail viewed from the underside. Measurements taken during fieldwork included 'tail tip difference' (TTD), which was the measurement between the shortest tail feather (T6) and the tip of the tail. 'Tail tip score' was recorded as the number of tail feather tips clearly visible (emergent ≥ 1mm beyond other feathers) on one side of the underside of the closed tail. In the schematic, the tail tip score for Willow Tit is 4 (T3, T4, T5 and T6) and for Marsh Tit the score is 2 (T5 and T6).



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182 Willow Tit biometrics

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A total of 179 handlings of 149 individual Willow Tits were made during 2007-2015 at several locations across England, including Berkshire (Combe Wood: 51°20'N 1°29'W, 17 birds), Lincolnshire (Market Rasen area: 53°21'N 0°14'W, 48 birds), Cheshire (Woolston Eyes: 53°23'N 2°31'W, 38 birds, and two birds at other sites), Greater Manchester and adjoining areas of Lancashire and Merseyside (within 9 km of Wigan: 53°32'N 2°37'W, 34 birds) and Yorkshire (Potteric Carr: 53°30'N, 1°6'W, Fairburn Ings: 53°44'N 1°19'W and Allerthorpe: 53°55'N 0°48'W, ten birds). Willow Tits were caught in all months of the year using cage traps or mist-nets and fitted with a BTO alloy ring, with Berkshire birds also being colour-ringed. As with Marsh Tits, ageing as first-years or adults was based on ringing history and plumage criteria (Laaksonen & Lehikoinen 1976, Broughton 2010), and 37 birds were sexed using behaviour or the appearance of a brood patch (BP) or cloacal protuberance (CP) during the breeding period (April-early July).

Measurement protocols followed those for Marsh Tits, with 20 birds (six of which were sexed) originally caught as first-years and later as adults being included in analyses of both age classes. Measurements taken comprised wing length, tail length, tail tip difference and tail tip score (Fig. 1), and body mass. Data were pooled from 11 ringers, including six trainers, with cross-checking for consistency between one author (RKB) and seven of the contributing ringers or their trainers.

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205 Statistical analyses

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207 Biometric summary statistics for both species included the variables given by Cramp & Perrins (1993), to allow for direct comparisons. Analyses of differences between age and 208 209 sex classes of Marsh Tits superseded previous work in Broughton et al. (2008) due to the availability of much larger sample sizes and range of biometrics. We used non-parametric 210 tests throughout to accommodate some non-normal distributions (assessed using Anderson-211 Darling tests) and to maintain consistency between all tests comparing groups. As birds 212 were caught and weighed throughout the day, reflecting the activity of typical ringers across 213 Britain, we made no adjustments to mass to account for time of day (as per Robinson 2015). 214

215 Marsh Tit wing lengths for birds of known age and sex were used in binomial logistic 216 regression, applying a logit link function and using age class as a factor. This was in order to 217 provide probability estimates for sexing birds in other samples, to indicate the sex of 218 unknown individuals or the proportion of males and females in different populations.

Willow Tit biometrics were compared between age and sex classes, and logistic regression was attempted in order to produce probability estimates for sexing birds using wing length, as per Marsh Tits. Wing length, mass and tail length measurements were compared with those of Marsh Tits, including use of discriminant analysis with species as the grouping factor. To investigate differences in tail shape, we calculated and compared the tail/wing index for Marsh and Willow Tits (Eck 2006), and the tail tip difference and tail tip score to test the separation methods of Redfern & Clark (2001) and du Feu & du Feu (1996).

All analyses were performed in Minitab 16, and usage followed methods in Dytham (2011). In particular, discriminant analysis in Minitab produces a linear discriminant function for each group, with the accuracy of classification given as a proportion of the sample. Unknown individuals can be classified with this reported accuracy by applying the function for each group and assigning the individual to the group which gives the highest value (Dytham 2011).

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234 RESULTS

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237 Marsh Tit biometrics

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Summary statistics for Marsh Tit biometrics are given in Table 1, with results of statistical comparisons between sexes. The DNA sexing of 55 birds (28 females and 27 males) agreed completely with the sex assigned from behavioural observations in the field, and so all birds were considered to be sexed correctly using the behaviour method.

Table 1 shows that the wing and tail lengths of males were significantly longer than those of females in all age classes, and adults of both sexes had longer wings than firstyears (Mann-Whitney tests, males: U = 29905.5, P < 0.001; females: U = 17057.0, P < 0.001). Adults also had longer tails than first-years (males: U = 3421.5, P < 0.001; females U 247 = 3776.5, P < 0.001). Individuals with longer wings generally had longer tails (males: 248 Spearman's rank-order correlation, $r_s = 0.76$, P < 0.001; females: $r_s = 0.59$, P < 0.001).

Male Marsh Tits were typically heavier than females (Table 1), but adults were not heavier than first-years (males: U = 21386.0, P = 0.947; females U = 13906.5, P = 0.948). Within all age and sex classes, heavier birds tended to have longer wing lengths (all groups: $r_{s} = 0.21-0.37$, P < 0.001-0.040).

Tarsus measurements of 59 birds (including 30 unsexed) showed a narrow range of values (17.8-19.4 mm, median = 18.6, mean = 18.5 ± 0.4 s.d.). The median tarsus length of males was longer than that of females (Table 1), but there was no correlation between tarsus and wing length in either sex (r_s = -0.02 and 0.25, P = 0.942 and 0.456 for males and females respectively).

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260 Estimating Marsh Tit population structure

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For adult Marsh Tits, a wing length division of 63 mm or less for females and 64 mm or more for males accurately sexed 96.7% of birds (99.1% of 107 females and 94.4% of 144 males). For first-years a division of \leq 62 mm for females and \geq 63 mm for males assigned 93.8% of birds to the correct sex (97.2% of 141 females and 91.0% of 167 males). Combining these statistics for both age classes sexed all 559 Marsh Tits with an overall accuracy of 95.0%, using the different wing length divisions for sexing adults and first-years.

The full output of the logistic regression models is given in Appendix 1, showing highly significant relationships between wing length, age and sex in Marsh Tits. Table 2 shows the probability of being male for an adult or first-year bird of a given wing length, with associated confidence intervals. Table 2 also gives the wing length frequencies and probability estimates for sexing Marsh Tits where age was unspecified (both age classes combined), which would correspond to unknown birds where age was not determined (e.g. a EURING age code of 2).

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277 Willow Tit biometrics

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279 The wings and tails of male Willow Tits tended to be longer than those of females, although not significantly so for first-years, and body weights showed no significant difference 280 between the sexes in any age class (Table 1). Wing lengths showed a substantial overlap 281 282 between the sexes (Table 3, age classes combined due to small sample sizes), and so 283 logistic regression of the wing lengths against sex did not produce good results, with a poor goodness-of-fit (deviance chi-square = 21.8, df = 6, P = 0.001) and poor measures of 284 285 association (73.1% concordant pairs between response variable and predicted probabilities). 286 As such, this approach was not pursued (full model output not shown).

When comparing biometrics of first-year and adult Willow Tits (sexes combined, Table 4) the adults had significantly longer wings (U = 7183.5, P = 0.048) and tails (U =4101.5, P = 0.012) than younger birds, with a near-significant difference in body mass (U =5089.5, P = 0.051) following the same trend, but the differences were small.

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293 Differences between Marsh and Willow Tits

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Similar proportions of males and females in samples of both species allowed sexes to be combined for analyses. These showed that Marsh Tits tended to have longer wings than Willow Tits, with proportionately shorter tails (tail/wing index), and were also heavier (Table 4). Marsh Tit wing lengths showed a strongly bimodal distribution, compared to unimodal Willow Tits (Fig. 2a). This reflected the greater overlap in wing lengths between male and female Willow Tits compared to Marsh Tits in both age groups.

Figure 2 illustrates the substantial overlap in wing length, tail length (Fig. 2b) and body mass (Fig. 2c) between species. Neverhteless, the wings of the smallest Willow Tits were 4 mm shorter than the smallest Marsh Tits, and they weighed 1.2 g less, whilst wing
lengths of the largest Marsh Tits were 4 mm longer than Willow Tits and they were 0.7 g
heavier, although the longest tails of Willow Tits were 3.5 mm longer than Marsh Tits (Table
4).

Figure 2. Biometrics of British Willow Tits (dotted lines) and Marsh Tits (solid lines) showing distributions of: a) wing lengths of 153 Willow Tits and 559 Marsh Tits, b) tail lengths of 118 Willow Tits and 253 Marsh Tits, and c) body mass of 124 Willow Tits and 477 Marsh Tits.



312 There were significant differences in tail shape between the two species, with a greater 'tail tip difference' for Willow Tits of all ages, compared to Marsh Tits (Table 4). 313 Despite this, the measurement range of tail tip difference overlapped for 71% of Marsh Tits 314 and 79% of Willow Tits. Nevertheless, for first-year birds, a division of 4 mm or more for 315 316 Willow Tits and 3.5 mm or less for Marsh Tits correctly identified 96% of both species. For adults, 95% of Willow Tits were correctly identified with a tail tip difference of \geq 5 mm, but 317 only 72% of Marsh Tits with a measurement of \leq 4.5 mm. Combining the statistics for both 318 age classes gave an overall accuracy of 89% for the tail tip difference method (44 errors 319 from 389 birds). 320

Willow Tits had significantly higher 'tail tip scores' than Marsh Tits, with generally more tail feathers visible on either side of the closed tail (underside) in all age classes (Table 5). Adults of both species had significantly higher scores than first-years (Willow Tit: U =264.0, P = 0.015; Marsh Tit, U = 5043.0, P < 0.001), but the range of scores overlapped completely at 2-5 for each species.

Discriminant analysis for separating Marsh and Willow Tits was most successful when using wing length and tail tip difference as predictor variables, assigning almost all birds to the correct species, particularly when adults and first-years were treated separately (Table 5).

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332 DISCUSSION
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335 Marsh Tit biometrics

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The results demonstrated that male Marsh Tits were generally larger and heavier than females in both age classes, and that adults were larger than juveniles. Individuals with long wings also had long tails and weighed more than smaller birds. These measurements represent the largest biometric dataset available for live Marsh Tits of the British subspecies, grouped by age and sex. The data come from a very small number of ringers with a high degree of consistency, and with accurate ageing and sexing derived from monitored populations in three English localities.

These results offer an alternative to the small sample of measurements from skins in Cramp & Perrins (1993), and also the measurements pooled from a large number of ringers in the BTO database (Robinson 2015), which shows unusual variability (du Feu & du Feu 2014). As is has been shown that is no regional variation in Marsh Tit biometrics across Britain (Broughton et al. 2016), our dataset can be considered as representative of this subspecies.

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352 Willow Tit biometrics

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Although our sample sizes for Willow Tits were relatively small, the data represent 354 the first material available for the British subspecies that is grouped by age and sex, and the 355 356 first statistical comparison between these groups. These tests revealed that, like Norwegian Willow Tits (Haftorn 1982, Hogstad 2011) and British Marsh Tits (this study), adult male 357 Willow Tits tended to have longer wings and tails than adult females, but, unexpectedly, this 358 was not the case amongst first-years or for body mass in any age class. More conforming to 359 expectations, adults had longer wings and tails than first-years overall, as in Norway (Haftorn 360 1982, Hogstad 2011) and for British Marsh Tits (this study). 361

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364 Estimating population structure

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Based on the large sample of Marsh Tit wing lengths in this study, the sexing probabilities derived from logistic regression could be used to estimate the sex ratio in other ringing data.

This could be achieved by simply calculating the percentage of birds of each wing length by the values for the appropriate age class in Table 2 to estimate the percentage of males in a sample. For example, of 125 first-years with a wing length of 61 mm, three birds could be expected to be male (2.4% of 125 = 3), leaving 122 females, whilst all adults of 60 mm would be expected to be female (0.0% probability of being male), and 99.8% of birds of 65 mm where age was unknown could be expected to be male.

As previous work has shown no variation in Marsh Tit wing length across the British range (Broughton et al. 2016), this probability approach could be applied to samples from any location. The associated confidence intervals indicate the precision of the estimates, and could be used to produce upper and lower estimates. This could test for a gender bias in other populations, which may result from differing survival rates between the sexes and could be a factor in the species' decline in abundance (Broughton & Hinsley 2015).

A caveat with using the sexing probabilities from Table 2 is that they are based on a large and relatively balanced sample of birds (56% males, 44% females). Applying these probabilities to very small samples from other populations could be misleading if one sex happens to be grossly over-represented by chance or capture method (e.g. catching at nestboxes, which may be heavily biased towards females). Larger samples, and a random sampling technique, will produce more reliable estimates.

For sexing individual Marsh Tits, the probabilities produced by the logistic regression 386 models had very narrow confidence intervals for most wing lengths, suggesting that most 387 individuals could be sexed with a very high degree of reliability (greater than 95%). For 388 example, Table 2 indicates that a first-year Marsh Tit with a wing length of 64 mm would 389 have a 99.7% probability of being male (with a confidence of 99.1-99.9%), whilst a bird of 59 390 mm wing length would be essentially certain to be female. The confidence intervals suggest, 391 however, that greater caution is required when sexing individuals with wing lengths of 62 mm 392 (first-years) or 63 mm (adults), which have much wider confidence intervals. 393

When using the more basic method of sexing Marsh Tits, i.e. the simple cut-off value for wing length, treating adults and first-years separately was an improvement on the method

of King & Muddeman (1995) and Broughton et al. (2008). These earlier studies 396 recommended separating females and males of all ages with a wing length division of 62/63 397 mm. Applied to our sample, this method still accurately sexed 93.4% of 559 birds, but by 398 using different divisions for adults (63/64 mm) and first-years (62/63 mm) we improved the 399 400 accuracy slightly to 95.0%. This compares to 93.5% accuracy for 230 birds in Broughton et al. (2008a), 92% for 89 birds in du Feu & du Feu (2014) and 96% for 50 birds in King & 401 Muddeman (1995). The probabilities given by the logistic regression models offer an 402 403 advantage by providing confidence intervals as a measure of the reliability of sexing an individual of a given wing length, which is particularly valuable for birds close to the cut-off 404 points. 405

406 The large degree of overlap between male and female Willow Tits in our relatively 407 small samples meant that biometric sexing was unreliable for this species. Only 74% of birds 408 were assigned to the correct sex if using the best wing length division of \leq 60 mm for 409 females and \geq 61 mm for males (94% of females but only 58% of males). These results were 410 surprising, as Markovets (1992) in Russia and Hogstad (2011) in Norway accurately sexed 99-100% of nominate race Willow Tits using a simple wing length division (63/64 mm in 411 412 Russia, 64/65 mm in Norway). Haftorn (1982), also in Norway, sexed 86% of Willow Tits using wing length. 413

Although Willow Tits can be sexed by assessing the brood patch and cloacal 414 protuberance, this method is only valid in spring (April-June, Markovets 1992) and can be 415 unreliable for non-breeding birds (pers. obs.). The ability to sex Willow Tits using biometrics 416 would be a valuable tool throughout the year, as, remarkably, Haftorn (1982) used 417 biometrics to show that a large proportion of skins in the University of Trondheim collection 418 had been wrongly sexed during autopsy. This shows the scientific value of recording 419 biometric measurements to validate other sexing methods. A larger sample of wing lengths 420 from birds of known age and sex could help to confirm whether a greater overlap between 421 422 the sexes is a feature of British Willow Tits, which may indicate a greater constraint on body 423 size in Britain compared to elsewhere.

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426 Separating Marsh and Willow Tits

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Our results show that British Marsh Tits are slightly more robust than Willow Tits, being larger and heavier (by a median 5-7%) with proportionately shorter tails. Tail shape was a useful feature for separating the species, particularly the measurement of 'tail tip difference' between the shortest and longest tail feathers, as proposed by Amann (1980) for Swiss birds, Abe & Kurosawa (1984) in Japan, and du Feu & du Feu (1996) and Scott (1999) in Britain.

434 Our results supported the findings of these earlier studies by showing that most British Marsh Tits (97%) had a tail tip difference of \leq 5 mm, with almost all Willow Tits (98%) 435 436 being \geq 4 mm, but the overlap of 4-5 mm included a large proportion of birds in our sample. The measured difference was generally greater for adults than first-years, as was also 437 shown by Amann (1980). Therefore, by treating the age classes separately we found that 438 most first-years (96%) could be separated with a sharp division of \leq 3.5 mm for Marsh Tits 439 440 and \geq 4 mm for Willow Tits. Adults showed more overlap than first-years, particularly Marsh Tits, but the division of 4.5/5 mm still identified most birds correctly. 441

Tail tip score, derived from the number of feather tips visible on one or other side of the closed tail, viewed on the underside, was less reliable than tail tip difference due to a significant proportion of adult Marsh Tits and some first-years having a graduated tail that was similar to a typical Willow Tit. Some birds also showed asymmetry in the tail, with one T6 feather noticeably shorter than the other. In these cases measurements taken from the longest T6 feather are recommended as a conservative approach.

The discriminant function using tail tip difference and wing length as predictors (Table 6) gave the best results for separating Marsh and Willow Tits with two simple measurements, identifying 95-99% of birds of either species (according to age class). The presence/absence of a pale mark at the base of the bill has previously been shown to 452 identify 99% of Marsh Tits and 94% of Willow Tits (Broughton et al. 2008b), and for 453 ambiguous birds the discriminant function based on wing length and tail tip difference now 454 offers an alternative of a similar accuracy. If these two tests are used together then the 455 chances of misidentification seem remote, but where they contradict then other supporting 456 features can be called upon, such as tail tip score and cheek pattern (Broughton 2009).

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459 Using biometrics in analyses

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The value of biometrics for identifying or sexing birds, or estimating population structure, ultimately relies on the accuracy of the data that are collected, as well as variation among the birds themselves (du Feu & du Feu 2014). For species such as Marsh and Willow Tits, where errors of 0.5-1 mm could make a difference in classification analyses, it is essential that data collection and recording are consistently accurate.

466 When detailed field studies on reasonable samples of Marsh Tits have been carried out in different parts of England, and measurements are consistent within each group of 467 468 ringers, then the results have been very similar: 92-96% of birds are sexed accurately using a simple wing length division of 62/63 mm (King & Muddeman 1995, Broughton et al. 2008, 469 470 du Feu & du Feu 2014, and the present study). It was highlighted by du Feu & du Feu (2014), however, that in the BTO ringing database only 71% of sexed Marsh Tits fitted this 471 pattern (Robinson 2015). This means that almost a third of the birds in the national dataset 472 would have been assigned to the wrong sex by the 62/63 mm wing length division, 473 compared to an error rate of just 4-8% in the detailed studies. As there is no regional 474 variation in Marsh Tit biometrics across Britain (Broughton et al. 2016), the most likely 475 explanation for the large discrepancy in the BTO database is variation in data quality. This 476 could result from measurement variability within or between ringers, or incorrect sexing from 477 misinterpretation of brood patch or cloacal protuberance (Redfern & Clark 2001). 478

Significant variability in data quality within the national dataset could undermine the use of Marsh Tit biometrics for the analysis of population structure. Similar issues may also exist for Willow Tit data, but a greater overlap in biometrics between the sexes could make these harder to detect. Although it may be possible to identify the more reliable series of ringing records for these species, perhaps by extracting those from sources of known reliability or by testing the measurement repeatability among recapture records, this case study underlines the need for accurate data collection in ringing schemes.

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Table 1. Biometric summary statistics (mean ± s.d. (median, range), *n*) for British Marsh Tits

and Willow Tits by sex and age (FY = first-years, Ad = adults, All = ages classes combined),

617 with comparisons of medians by Mann-Whitney *U*-tests.

	Female	Male	U	Р
Marsh	Tit wing length (mm)			
FY	60.8 ± 0.8 (61.0, 59.0-63.0), 141	63.7 ± 0.9 (64.0, 60.0-66.0), 167	10431.0	<0.001
Ad	61.7 ± 1.0 (62.0, 59.0-65.0), 107	64.8 ± 0.9 (65.0, 61.0-67.0), 144	5997.0	<0.001
All	61.2 ± 1.0 (61.0, 59.0-65.0), 248	64.2 ± 1.1 (64.0, 60.0-67.0), 311	32823.5	<0.001
Marsh	Tit tail length (mm)			
FY	49.9 ± 1.4 (50.0, 46.5-55.5), 63	52.3 ± 1.4 (52.0, 49.0-57.5), 71	2468.5	<0.001
Ad	50.8 ± 1.1 (51.0, 49.0-53.5), 53	53.8 ± 1.0 (54.0, 51.0-56.0), 69	1539.5	<0.001
All	50.3 ± 1.3 (50.0, 46.5-55.5), 116	53.1 ± 1.5 (53.0, 49.0-57.5), 140	8188.5	<0.001
Marsh	Tit body mass (g)			
FY	10.4 ± 0.3 (10.4, 9.6-11.3), 125	11.1 ± 0.4 (11.1, 9.9-12.1), 152	9577.0	<0.001
Ad	10.4 ± 0.4 (10.5, 9.5-11.2), 96	11.1 ± 0.4 (11.1, 10.0-11.9), 129	6087.5	<0.001
All	10.4 ± 0.3 (10.4, 9.5-11.3), 221	11.1 ± 0.4 (11.1, 9.9-12.1), 281	30878.0	<0.001
Marsh	Tit tarsus length (mm)			
All	18.4 ± 0.4 (18.4, 17.8-18.9), 11	18.8 ± 0.3 (18.7, 18.4-19.4), 18	102.5	<0.001
Willow	Tit wing length (mm)			
FY	59.3 ± 2.1 (59.5, 56-63), 8	60.0 ± 1.6 (60.0, 58-62), 10	65.5	0.364
Ad	58.9 ± 1.4 (59.5, 57-60), 10	61.2 ± 1.5 (62.0, 58-63), 14	73.0	0.002
All	59.1 ± 1.7 (59.5, 56-63), 18	60.7 ± 1.6 (61.0, 58-63), 24	272.0	0.003
Willow	Tit tail length (mm)			
FY	50.3 ± 1.8 (51.0, 48.0-52.5), 6	51.7 ± 3.9 (50.0, 49.0-60.0), 7	39.0	0.718
Ad	50.0 ± 2.5 (50.0, 46.0-53.0), 7	52.8 ± 1.9 (53.0, 49.0-55.0), 11	42.0	0.028
All	50.1 ± 2.1 (51.0, 48.0-53.0), 13	52.4 ± 2.8 (52.0, 49.0-60.0), 18	153.0	0.028
Willow	Tit body mass (g)			
FY	9.8 ± 0.6 (9.9, 8.9-10.4), 7	9.9 ± 0.5 (9.7, 9.2-10.6), 9	56.0	0.750
Ad	10.1 ± 0.8 (10.2, 8.5-11.0), 8	10.2 ± 0.6 (10.1, 9.3-11.2), 12	95.5	0.831

	All	9.9 ± 0.7 (9.9, 8.5-11.0), 16	10.1 ± 0.6 (9.9, 9.2-11.2), 21	293.5	0.759
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Table 2. Frequency distribution of Marsh Tit wing lengths for first-years, adults and all birds (age unspecified), showing the number of females (F) and males (M) of a given wing length in each category. The probability of being male (P(M)) for an individual of a given wing length is shown as a percentage, with the associated 95% confidence intervals (CI).

WingFM $P(M)$ FM $P(M)$ (mm)(n)(n)(%)95% CI(n)(n)(%)95% CI59600.00.0200.00.0604010.10.0-0.3900.00.0617002.40.8-7.22910.10.1-0.262211437.315.7-65.55002.41.3-4.46345193.582.0-97.916737.324.2-52.4	F (<i>n</i>) 8 49 99	M (<i>n</i>) 0 1 1	P(M) (%) 0.0 0.1 1.5	95% CI 0.0 0.1-0.2 0.9-2.4
(mm)(n)(n)(%) 95% CI(n)(n)(%) 95% CI59600.00.0200.00.0604010.10.0-0.3900.00.0617002.40.8-7.22910.10.1-0.262211437.315.7-65.55002.41.3-4.46345193.582.0-97.916737.324.2-52.4	(<i>n</i>) 8 49 99	(<i>n</i>) 0 1 1	(%) 0.0 0.1 1.5	95% CI 0.0 0.1-0.2 0.9-2.4
59 6 0 0.0 0.0 2 0 0.0 0.0 60 40 1 0.1 0.0-0.3 9 0 0.0 0.0 61 70 0 2.4 0.8-7.2 29 1 0.1 0.1-0.2 62 21 14 37.3 15.7-65.5 50 0 2.4 1.3-4.4 63 4 51 93.5 82.0-97.9 16 7 37.3 24.2-52.4	8 49 99	0 1 1	0.0 0.1 1.5	0.0 0.1-0.2 0.9-2.4
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63 4 51 93 5 82 0-97 9 16 7 37 3 24 2-52	17	14	17.8	11.8-25.9
	5 20	58	75.7	65.8-83.5
64 0 75 99.7 99.1-99.9 0 40 93.5 88.6-96.4	4 0	115	97.8	96.5-98.6
65 0 24 100.0 1 69 99.7 99.5-99.3	81	93	99.8	99.8-99.9
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67 0 0 100.0 100.0 0 2 100.0 100.0	0	2	100.0	100.0

Table 3. Frequency distribution of wing lengths for 18 female and 24 male Willow Tits, with

	Wing length	Fe	male	Ν	lale
	(mm)	n	%	n	%
	56	1	100.0	0	0.0
	57	4	100.0	0	0.0
	58	0	0.0	4	100.0
	59	4	80.0	1	20.0
	60	8	62.0	5	38.0
	61	0	0.0	4	100.0
	62	0	0.0	8	100.0
	63	1	33.0	2	67.0
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the percentage of birds of each sex for a given wing length.

Table 4. Comparisons between the biometrics of British Marsh Tits and Willow Tits, with results of Mann-Whitney *U*-tests of medians. Birds are grouped by age class (FY = first-year, Ad = adult, All = age classes combined). Tail tip difference refers to the measurement between the tips of the shortest and longest tail feathers, and tail tip score refers to the number of tail feather tips visible on either side of the underside of the closed tail.

	Marsh Tit	Willow Tit	U	Р
Wing	length (mm)			
FY	62.4 ± 1.7 (63.0, 59.0-66.0) 308	59.5 ± 1.7 (60.0, 55.0-63.0) 102	75296.0	< 0.001
Ad	63.5 ± 1.8 (64.0, 59.0-67.0) 251	60.1 ± 1.6 (60.0, 57.0-63.0) 51	43282.5	< 0.001
All	62.9 ± 1.8 (63.0, 59.0-67.0) 559	59.7 ± 1.7 (60.0, 55.0-63.0) 153	233048.5	< 0.001
Tail le	ength (mm)			
FY	51.2 ± 1.8 (51.0, 46.5-57.5) 134	50.6 ± 2.6 (50.5, 45.0-61.0) 77	15133.5	0.028
Ad	52.5 ± 1.9 (53.0, 49.0-56.0) 122	51.6 ± 2.5 (52.0, 46.0-61.0) 40	10634.0	0.007
All	51.8 ± 2.0 (52.0, 46.5-57.5) 256	50.9 ± 2.6 (51.0, 45.0-61.0) 117	51768.0	< 0.001
Tail/w	ving index			
FY	0.82 ± 0.02 (0.82, 0.77-0.91) 134	0.85 ± 0.04 (0.85, 0.77-1.00) 76	10998.0	< 0.001
Ad	0.83 ± 0.01 (0.83, 0.78-0.86) 122	0.86 ± 0.03 (0.85, 0.80-1.00) 39	8200.0	< 0.001
All	0.83 ± 0.02 (0.83, 0.77-0.91) 256	0.85 ± 0.03 (0.85, 0.77-1.00) 115	38354.5	< 0.001
Tail ti	p difference (mm)			
FY	2.8 ± 0.6 (3.0, 1.5-5.0) 200	5.2 ± 1.0 (5.0, 3.0-8.0) 76	20320.5	< 0.001
Ad	4.2 ± 0.9 (4.0, 2.0-6.0) 71	6.2 ± 1.1 (6.0, 4.0-9.0) 42	2793.5	< 0.001
All	3.2 ± 0.9 (3.0, 1.5-6.0) 271	5.6 ± 1.2 (5.5, 3.0-9.0) 118	38624.0	< 0.001
Tail ti	p score			
FY	2.1 ± 0.3 (2, 2-3) 107	3.4 ± 0.7 (4, 2-4) 23	5967.5	< 0.001
Ad	2.9 ± 0.7 (3, 2-5) 47	4.3 ± 0.5 (4, 4-5) 8	1156.0	< 0.001
All	2.4 ± 0.6 (2, 2-5) 154	3.6 ± 0.7 (4, 2-5) 29	12460.5	< 0.001
Body	mass (g)			
FY	10.8 ± 0.5 (10.7, 9.6-12.1) 277	9.9 ± 0.6 (9.9, 8.3-11.4) 86	58897.5	< 0.001
Ad	10.8 ± 0.5 (10.8, 9.5-11.9) 225	10.2 ± 0.6 (10.2, 8.5-11.4) 40	32468.5	< 0.001

All	10.8 ± 0.5 (10.8, 9.5-12.1) 502	10.0 ± 0.6 (10.0, 8.3-11.4) 126	178884.5	< 0.001
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Table 5. Linear discriminant function for separating Marsh Tits and Willow Tits of different age classes using measurements of wing length and tail tip difference (TTD, the measurement between the longest and shortest tail feathers on the underside of the tail). Identification is determined by inserting the measurements of wing length and tail tip difference for an individual into the equations for the relevant age class, and then assigning the bird to whichever species gives the highest value from the equation. The functions are based on measurements of 271 Marsh Tits (200 first-years, 71 adults) and 117 Willow Tits (76 first-years, 41 adults), and probabilities of correct identification are expressed as the % of birds in the sample that were correctly assigned to species.

Species	Lincor discriminant function	% identified	Overall %
Species	Linear discriminant function	accurately	accuracy
First-years			
Marsh Tit	(wing length x 24.16) + (TTD x 0.31) – 753.35	99.0	07.0
Willow Tit	(wing length x 22.89) + (TTD x 5.04) – 694.57	94.7	97.8
Adults			
Marsh Tit	(wing length x 23.11) + (TTD x –1.05) – 733.36	95.8	<u></u>
Willow Tit	(wing length x 21.61) + (TTD x 1.49) – 654.21	95.1	95.5
All ages			
Marsh Tit	(wing length x 22.74) + (TTD x –6.88) – 701.68	96.7	00.4
Willow Tit	(wing length x 21.26) + (TTD x –3.77) – 624.76	95.7	96.4

723 Appendix 1

- a) Output from Minitab 16 for binomial logistic regression of Marsh Tit wing length versus
- sex, with age class (FY = first-year, otherwise adult) as a factor, using a logit link function.

						95%	% CI
Predictor	Coef	SE Coef	Ζ	Р	Odds Ratio	Lower	Upper
Constant	-201.53	20.04	-10.06	0.000			
Marsh Tit wing	3.19	0.32	10.06	0.000	24.30	13.05	45.25
Marsh Tit age							
First-year	3.19	0.54	5.95	0.000	24.35	8.50	69.76

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727 Log-Likelihood = -78.08

- 728 Test that all slopes are zero: G = 611.67, df = 2, P < 0.001
- Goodness-of-fit test: Deviance chi-square = 23.45, df = 14, P = 0.053
- 730

b) Output from Minitab 16 for binomial logistic regression of Marsh Tit wing length versus

sex, (age is unspecified), using a logit link function.

-							95% CI	
	Predictor	Coef	SE Coef	Ζ	Р	Odds Ratio	Lower	Upper
-	Constant	-607.00	15.41	-10.84	0.000			
	Marsh Tit wing	2.67	0.25	10.84	0.000	14.42	8.90	23.27

- 734 Log-Likelihood = -104.28
- 735 Test that all slopes are zero: G = 559.26, df = 1, P < 0.001
- Goodness-of-fit test: Deviance chi-square = 11.62, df = 7, P = 0.114